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☑ 11. 6277607 . 24 May 99; 21 Aug 01. High specificity primers, amplification methods and kits. Tyagi; Sanjay, et al. 435/91.2; 435/6 435/91.1 436/94 536/23.1 536/24.3 536/24.33. C12P019/34 C12Q001/68 G01N033/00 C07H021/02 C07H021/04.

☐ 12. 6143901 . 21 Apr 97; 07 Nov 00. Complex formation between dsDNA and pyrrole imidazole polyamides. Dervan; Peter B.. 548/312.4; 435/75. C07D231/02 C07D403/02 C12P019/44.

☐ 13. 6090947 . 26 Feb 96; 18 Jul 00. Method for the synthesis of pyrrole and imidazole carboxamides on a solid support. Dervan; Peter B., et al. 548/312.4; 536/22.1 536/23.1 536/25.3 536/25.33 536/25.6 536/26.1 548/312.1 548/312.7 548/313.1 548/314.7 548/334.5 548/557. C07D231/02 C07D403/02 C07D233/04 C07N019/00 C07N021/02 C07N021/04.

☐ 14. 6083692 . 03 Jun 96; 04 Jul 00. Method of detecting the presence and measuring the quantity of biological polymers. Satishchandran; C., et al. 435/6; 435/196 436/94 536/23.1. C12Q001/68 C12N009/14 G01N033/52 C07H021/04.

☐ 15. 6025140 . 16 Jul 98; 15 Feb 00. Membrane-permeable constructs for transport across a lipid membrane. Langel; Ulo, et al. 435/6; 435/325 530/323 536/23.1 536/24.5. C07H021/04 C12Q001/68 C12N015/85.

☐ 16. US 20020034754 A1 . Oligonucleotide probes comprise a fluorophore linked to one end and a minor groove binding agent and a fluorescence-quenching azo dye linked to the other end. DEMPCY, R O, et al. C07H021/04 C09B029/00 C12Q001/68.

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L3: Entry 11 of 16 File: USPT Aug 21, 2001

DOCUMENT-IDENTIFIER: US 6277607 B1
TITLE: High specificity primers, amplification methods and kits

Abstract Text (1):
For nucleic acid amplification including extension of primers by a DNA polymerase, high specificity primers are provided. The primers include a type of hairpin structure in which a single-stranded loop separates complementary 3' and 5' arms and in which the loop and the 3' arm are complementary to the target nucleic acid. Amplification methods, assays and kits including such primers are included in the invention.

Brief Summary Text (1):
This invention relates to nucleic acid detection that includes amplification of target sequences.

Brief Summary Text (3):
Amplification utilizing DNA primers and a DNA polymerase is a well known technique for detecting nucleic acid target sequences. Methods for exponential amplification include the polymerase chain reaction (PCR), strand displacement amplification (SDA), nucleic acid sequence-based amplification (NASBA), transcription-mediated amplification (TMA), and rolling-circle amplification (RCA). Among numerous DNA polymerases commonly used are Thermus aquaticus DNA polymerase and reverse transcriptase. The design of linear DNA oligonucleotide amplification primers is generally accomplished with the acid of a computer program designed for that purpose. Among the available programs that can be utilized are PRIDE (Haas et al. 1998), OLIGO (Rychlik et al. 1989), OSP (Hilber et al. 1991), Primo (Li et al. 1997) and Primer Master (Proutski et al. 1996).

Brief Summary Text (5):
One major application for target-amplification methods is in vitro diagnostics. In diagnosing pathological conditions by nucleic acid-based techniques, a common situation is that a unique nucleic acid sequence from a pathogen is a rare component of the total nucleic acid in a clinical sample. For example, the genomic DNA of the malarial parasite is a very small fraction of the total DNA that is extracted from a patient's blood. Amplification of rare pathogenic target sequences is an effective means for detection in some cases, because primers can be designed that successfully ignore the abundant human sequences sufficiently for diagnostic purposes. However, there are many situations in which a rare target sequence is very similar to an abundant sequence, differing in some cases by only a single nucleotide. For example, certain human cancers are characterized by an alteration at just one nucleotide position in a gene (Lengauer et al., 1998). To detect these cancers at an early stage, or to detect their remnants after surgical removal of a tumor, it is necessary to detect the presence of a rare sequence that differs from an abundant sequence by only a single nucleotide. When a sequence that indicates the presence of cancer is rare, the difficulty of detecting that sequence is sometimes referred to as the "minimal residual disease problem." A similar problem arises when the emergence of a drug-resistant bacterium or virus needs to be detected as early as possible when a patient is being treated with a drug, because a number of drug-resistance genotypes are characterized by a single nucleotide substitution in a pathogenic sequence. For applications such as those described above, simple target amplification is not effective, because the primers cannot sufficiently distinguish between two sequences that differ from each other by only a single nucleotide substitution.

Brief Summary Text (6):
Two approaches have been used to address this problem. The first is to design one of the two oligonucleotide primers that are needed for amplification to bind to the target at a sequence that encompasses the site of the nucleotide substitution. If the primer is perfectly complementary to its intended target sequence, then a

primer-target hybrid will form, leading to the generation of amplified copies of the target nucleic acid sequence. The hope is that if a nucleotide substitution is present, then the mismatched primer-target hybrid will not form, resulting in an inability to generate amplified copies of the nucleic acid sequence. However, this dichotomy does not work well in practice, and both the mutant and the wild-type templates result in amplification. The products of amplification of perfect and mismatched targets (the "amplicons") are indistinguishable from one another. Even if only mismatched target sequences are present in the sample, the primer will occasionally initiate DNA synthesis on the mismatched target sequence. Because the resulting product contains a perfect complement of the primer sequence, exponential amplification of this initial product occurs at a rapid rate. The second approach that is used to detect mutations in a target sequence is to utilize primers that bind outside the sequence that might contain a mutation, so that the sequence that contains the site of the mutation becomes a part of the resulting amplicons. Additional hybridization probes are then used to determine if the mutation is present within the amplicons. The proportion of amplicons containing a mutation is a measure of the relative amount or absolute amount of the mutation in the starting sample. Although this approach works well in many situations (Tyagi et al. 1996, Tyagi et al. 1998), it has a sensitivity limitation: if the mutant amplicons are less than a few percent of all the amplicons, they cannot be detected.

Brief Summary Text (10):
One aspect of the invention is an improvement in the sensitivity of assays that detect target nucleic acids that contain a single nucleotide substitution within a population of much more abundant wild-type nucleic acids, enabling detection at levels below a few percent.

Brief Summary Text (12):
Another aspect of the invention is that it enables the determination of the fraction of a nucleic acid population that is mutant and the fraction that is wild type, particularly when the fraction is very small or very large.

Brief Summary Text (16):
This invention includes oligonucleotide primers for nucleic acid amplification. When not bound to target, primers according to this invention form a particular type of hairpin structure in which the 3' terminal region of the primer is hybridized to the 5' terminal region of the primer to form a double-stranded stem. Only the central region of the primer is single stranded and available for initial hybridization to a complementary target, a process sometimes referred to as "nucleation". This invention also includes amplification methods and assays that utilize such primers, and kits for performing such assays. These methods and assays reduce false amplicon synthesis that limit existing methods and assays.

Brief Summary Text (18):
Amplification reactions and assays according to this invention utilize at least one hairpin primer according to the invention. Exponential amplification reactions and assays (for example, the polymerase chain reaction) utilize a pair of primers, sometimes referred to as "forward" and "reverse" primers, one of which is complementary to a nucleic acid strand and the other of which is complementary to the complement of that strand. Where a pair of amplification primers is used, either one or both are hairpin primers according to this invention.

Brief Summary Text (19):
Assays according to this invention may utilize any detection method for detecting amplicons. Such methods include gel electrophoresis, intercalating dyes, minor groove binding dyes, fluorescence polarization, mass spectrometry and labeled detection probes. Detection may be end point, that is, carried out when amplification is completed, or real time, that is, carried out during the amplification process. Real-time probe-based detection methods include 5' nuclease assays (Gelfand et al., 1996; Livak et al., 1996) and molecular beacon assays (Tyagi et al., 1996; Tyagi et al., 1997; Tyagi et al., 1998). Alternatively, primers according to this invention can be labeled with interactive fluorescent label pairs such as two fluorophores or a fluorophore and a non-fluorescent quencher, such that a change in fluorescence signal indicates the presence of primers that have been extended and, thus, the presence of a target for the primers in a sample. Interaction between labels may be by fluorescence

resonance energy transfer (FRET), by touching, or both. Assays utilizing labeled primers according to this invention can be real-time assays as well as end-point assays.

Detailed Description Text (4):
Because the 3' portion of the primer is in a double-stranded state, the generation of false amplicons by primer-dimer formation or mis-priming of 3' ends is prohibited to a very large extent. If the sample contains a nucleic acid differing from target 5 by a nucleotide that is not complementary to the sequence in loop 3, the loop cannot bind to that nucleic acid and 3' arm 4 cannot anneal to the nucleic acid and initiate DNA synthesis. Consequently, the presence of target molecules (for example, either wild-type or mutant DNA template molecules, or wild-type or mutant RNA template molecules) in the sample results in amplicon synthesis, whereas the presence of molecules differing from the target sequence by as little as a single nucleotide substitution either does not result in amplicon synthesis, or if the primer happens on very rare occasions to bind to the mismatched template and initiate amplicon synthesis, the synthesis of the amplicons will be significantly delayed.

Detailed Description Text (5):
Referring to FIG. 1, it will be seen that in the embodiment depicted there, the terminal nucleotides of the primer are complementary and form part of stem. We refer to them as "arms". This is also true for the embodiment shown in FIG. 5. This is our preferred construction utilized in the several examples described below. However, it is within the scope of the invention that non-complementary nucleotides be included within the stem, which will reduce the strength of the stem, or included as non-complementary terminal nucleotides or the stem, for example, a single-stranded overhang. The latter presents a danger of false priming, so any overhang must be incapable of nucleation with other nucleic acid sequences so as to provide a starting point for branch migration. In primers according to this invention nucleation is by annealing of the loop sequence to the target. It will be understood that as used in this application and the appended claims "stem" and "complementary 3' and 5' arm sequences" are sufficiently broad to encompass the above variants.

Detailed Description Text (7):
Hairpin primers of the present invention can contain deoxyribonucleotides, ribonucleotides, peptide nucleic acids (PNA), other modified nucleotides, or combinations of these. Modified nucleotides may include, for example, 2'-O-methylribonucleotides or nitropyrole-based nucleotides. Modified internucleotide linkages may also be included, for example phosphorothioates. Using modified nucleotides in the 5' arm provides a way to adjust the strength of the stem. Other advantages of using such modifications for a particular application will be apparent to persons familiar with the art. In particular, hairpin primers according to this invention that are constructed from modified nucleotides may form stronger hybrids than if the primers were constructed from deoxyribonucleotides, thus enabling structured target sequences (such as those that occur in messenger RNAs) to be more easily accessed.

Detailed Description Text (12):
Hairpin primers of this invention are useful in a number of nucleic acid amplification processes that employ primers, including polymerase chain reactions (PCR), strand displacement amplification (SDA), nucleic acid sequence-based amplification (NASBA), transcription-mediated amplification (TMA), and rolling-circle amplification (RCA). The high specificity and consequent low mis-priming significantly and detectably delays signal from sequences other than perfectly complementary targets. In thermal cycling amplifications, this delay is manifested in a later threshold cycle: in isothermal amplifications, in a later time for detectable signal to arise.

Detailed Description Text (13):
Nucleic acid amplification assays that utilize hairpin primers of the present invention can be designed so that the amplicons are detected by conventional methods at the end of the reaction, such as by polyacrylamide gel electrophoresis, or the products can be detected in real time during amplification using conventional detection methods. A variety of real-time detection methods, including the use of intercalating dyes and probe-based methods, can be employed (reviewed by Zubritsky, 1999), or the fluorescence of labeled hairpin primers themselves can be utilized, as

described in Example 3 below.

Detailed Description Text (30):
In order to demonstrate that hairpin primers according to this invention can be labeled in such a way that their fluorescence increases upon their incorporation into amplicons, we synthesized a hairpin primer 51 whose sequence is shown in FIG. 5. The primer had a loop sequence eight nucleotides long, a stem seven nucleotides long, resulting in a target-complementary sequence 15 nucleotides long. A DABCYL quencher moiety 52 was covalently linked to the fifth nucleotide from the 3' end of the oligonucleotide (which was a thymidine nucleotide) via a hexalkyl spacer 53 and a fluorescein moiety 54 was covalently linked to the 5' end of the oligonucleotide, also via a hexalkyl spacer 55. The methods described by Tyagi and Kramer (1996) were utilized for the construction of this primer. We found that the fluorescence of the fluorophore 54 in this primer was quenched when the primer was incubated in the absence of target nucleic acids. When this primer was incubated in the presence of perfectly complementary targets, its fluorescence increased substantially. This primer was utilized in two polymerase chain reactions carried out under the same conditions described for Example 1, with the exception that the intercalating fluorescent dye, SYBR Green, was omitted. The first reaction was initiated with 20,000 template molecules that contained a target sequence that was perfectly complementary to the loop sequence and 3' arm sequence of the hairpin primer, whereas the second reaction contained no template DNA. The intensity of fluorescein fluorescence was monitored in real-time during each annealing stage of the polymerase chain reaction, and the results are plotted for both reactions as a function of the number of thermal cycles completed in FIG. 6. The results show that the fluorescence of the reaction mixture initiated with template DNA, curve 61, increases dramatically when the polymerase chain reaction enters the linear phase. However, the fluorescence of the reaction that did not contain any template DNA, curve 62, remained low throughout the course of the polymerase chain reaction. A comparison of the fluorescence intensity of the two reaction mixtures at the end of the amplification process indicates that their fluorescence is so different that the reaction initiated in the absence of template DNA could easily be distinguished from the reaction initiated with template DNA simply by illuminating the reaction tubes with an ultraviolet lamp. We also synthesized hairpin primers according to this invention that utilized a different labeling scheme from the one mentioned above, and we tested the resulting labeled hairpin primers. In this alternative labeling scheme, the 3' end of the primer was a thymidine nucleotide that was covalently linked to a fluorescein moiety via a hexalkyl spacer that was linked to the nucleotide, rather than being linked to the 3'-hydroxyl group of the deoxyribose moiety. This primer contained a 5'-DABCYL moiety. The primer was well quenched and its fluorescence was restored when it was incorporated into amplicons. Despite the linkage of the fluorescein moiety to the 3' nucleotide, this primer was extended normally by incubation with DNA polymerase in an amplification reaction.

Detailed Description Text (33):
The design of the first and second hairpin primers is illustrative of two design points. For the first hairpin primer we noted that a loop eight nucleotides long and a stem six nucleotides long could be obtained by adding only three nucleotides to the 5' terminus of the first conventional primer. That was possible in this particular instance, because of fortuitous complementarily. Note that three nucleotides in the 5' arm are there nucleotides at the 5' end of the first conventional primer. For the second hairpin primer the 5' arm includes six added nucleotides. However, in this instance note that the loop, thirteen nucleotides long, is outside the preferred range of 5-12 nucleotides. In this instance we were not attempting to discriminate against a single nucleotide substitution. Four polymerase chain reactions were performed, two containing the conventional primers, and two containing the hairpin primers. One of the reactions in each pair was initiated with target template DNA, whereas the other reaction in the pair did not contain any template DNA. The progress of these reactions was monitored using the fluorescent intercalating dye, SYBR Green, to label any amplicons that were generated in the course of the amplification reactions. The changes in fluorescence intensity that were observed during the course of the amplification reactions is shown in FIG. 7. The results show that the reaction that contained the first and second conventional primers and 20,000 template molecules, curve 71, became positive after 16 thermal cycles had been completed, and the reaction that contained those conventional primers and no template DNA, curve 73, became positive after 29 thermal cycles had been completed, indicating that false amplicons

were generated in the course of the reaction. A subsequent analysis of these amplicons by polyacrylamide gel electrophoresis showed that legitimate amplicons were produced in the first reaction, whereas false amplicons of an unexpected size were produced in the second reaction. We believe the false amplicons were primer-dimers. However, when the first and second hairpin primers were used in place of the conventional primers, the reaction initiated with 20,000 target template molecules, curve 72, became positive after 17 thermal cycles had been completed, but the reaction that did not contain any template molecules, curve 74, never became positive. Subsequent analysis of the amplicons by polyacrylamide gel electrophoresis confirmed that the first reaction generated the expected amplicons, and the second reaction did not generate any amplicons. These results confirm that hairpin primers of the present invention are useful in solving the problem of false amplicon synthesis during amplification reactions. The reason that hairpin primers suppress the synthesis of false amplicons is that only the sequence in the loop is available for initiating the primer-template hybrid, and the presence of a hybridization sequence in a hairpin loop renders the interaction between the primer and the target nucleic acid much more specific than the interaction that occurs when a conventional linear primer hybridizes to a target nucleic acid. Moreover, the structure of hairpin primers is such that the sequences that are present in the arms of the primer do not participate in the initial hybridization of the primer to the target nucleic acid. Consequently, the binding of one primer to another during an amplification reaction (which can create undesirable amplifiable primer-dimers when conventional linear primers are used) is much less likely to occur when hairpin primers are used.

Detailed Description Text (36):
Haas, S., Vingron, M., Poustka, A., and Wiemann, S. (1998) Primer design for large scale sequencing. Nucleic Acids Res. 26, 3006-3012.

Detailed Description Text (40):
Moran et. al., (1996) Nucleic Acids Res. 24, 2044-2052.

Detailed Description Text (41):
Nazarenko, I. A., Bhatnagar, S. K., and Hohman, R. J. (1997) A closed tube format for amplification and detection of DNA based on energy transfer. Nucleic Acids Res. 25, 2516-2521.

Detailed Description Text (42):
Newton, C. R., Graham, A., Heptinstall, L. E., Powell, S. J., Summers, C., Kalsheker, N., Smith, J. C., and Markham, A. F. (1989) Analysis of any point mutation in DNA. The amplification refractory mutation system (ARMS). Nucleic Acids Res. 17, 2503-2516.

Other Reference Publication (4):
Haas et al., "Primer Design for Large Scale Sequencing", Nucleic Acids Research, 26(12):3006-3012, 1998.

Other Reference Publication (11):
Moran et al., "Non-Hydrogen Bonding `Terminator` Nucleosides Increase the 3'-End Homogeneity of Enzymatic RNA and DNA Synthesis", Nucleic Acids Research, 24:2044-2052, 1996.

Other Reference Publication (12):
Nazarenko et al., "A Closed Tube Format for Amplification and Detection of DNA Based on Energy Transfer", Nucleic Acids Research, 25(12):2516-2521, 1997.

Other Reference Publication (13):
Newton et al., "Analysis of any Point Mutation in DNA. The Amplification Refractory Mutation System (ARMS)", Nucleic Acids Research, 17(7):2503-2516, 1989.

CLAIMS:

1. A process for nucleic acid amplification comprising the extension by a DNA polymerase of at least one hairpin oligonucleotide primer comprising a 3' arm sequence, a 5' arm sequence and a single-stranded loop sequence separating said arm sequences, said 3' arm sequence and said loop sequence both being perfectly complementary to a selected priming region of a target nucleic acid strand, and said

arm sequences having sufficient complementarity to one another to form a double-stranded stem hybrid in the absence of the target strand, wherein said primer hybridizes to said target strand overcoming the stem hybrid, wherein said stem hybrid is sufficiently strong that hybridization of the loop sequence to a sequence of the length of the loop and perfectly complementary to the loop sequence does not cause dissociation of the stem, and wherein said 3' arm sequence contains insufficient nucleotides that are not complementary to said 5' arm sequence to nucleate hybridization of the primer to the target.

2. The process according to claim 1 wherein said nucleic acid amplification is selected from the group consisting of a polymerase chain reaction (PCR), a strand displacement reaction (SDA), a nucleic acid sequence-based amplification (NASBA), a transcription-mediated amplification(TMA), and a rolling-circle amplification (RCA).

3. The process according to claim 2 wherein said nucleic acid amplification process includes a pair of primers.

6. The process according to claim 1 including real-time detection, wherein said at least one primer additionally comprises interactive fluorescent label moieties attached to the 3' and 5' arm sequences, whereby incorporation of the primer into a double-stranded nucleic acid detectably alters the fluorescence emitted by said label moieties.

13. The process according to claim 12 wherein said primer additionally comprises interactive fluorescent label moieties attached to the 3' and 5' arm sequences, whereby incorporation of the primer into a double-stranded nucleic acid detectably alters the fluorescence emitted by said label moieties.

15. The process according to claim 1 wherein said nucleic acid amplification process includes a pair of primers.

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☐ 2. 6277607 . 24 May 99; 21 Aug 01. High specificity primers, amplification methods and kits. Tyagi; Sanjay, et al. 435/91.2; 435/6 435/91.1 436/94 536/23.1 536/24.3 536/24.33. C12P019/34 C12Q001/68 G01N033/00 C07H021/02 C07H021/04.

☑ 3. 6083692 . 03 Jun 96; 04 Jul 00. Method of detecting the presence and measuring the quantity of biological polymers. Satishchandran; C., et al. 435/6; 435/196 436/94 536/23.1. C12Q001/68 C12N009/14 G01N033/52 C07H021/04.

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L2: Entry 3 of 3 File: USPT Jul 4, 2000

DOCUMENT-IDENTIFIER: US 6083692 A
TITLE: Method of detecting the presence and measuring the quantity of biological polymers

Brief Summary Text (6):
DNA and RNA based therapeutic and prophylactic agents are becoming a growing field of product development. Advances in the understanding of biology has led to the development of several fields of endeavor, such as nucleic acid vaccines, antisense compounds and gene therapy, that use DNA- and RNA-based agents.

Brief Summary Text (7):
One problem associated with use of these types of agents is that they can be contaminated with small quantities of molecules whose presence is undesirable. For example, recombinant protein products may be contaminated with plasmid and/or chromosomal DNA. Plasmid DNA usually contains antibiotic resistance genes. The possibility of contamination of protein products by plasmid DNA therefore raises safety and public health concerns which must be addressed. Similarly, nucleic acid-based agents such as plasmid-based vaccines can be contaminated with protein or linear DNA, the administration of either as contaminants being undesirable. Antisense compounds may similarly be contaminated with proteins. The presence of contaminants in nucleic acid-based agents raises safety issues which must be addressed.

Brief Summary Text (8):
In addition to the detection of contaminants in samples of material, modern molecular biology and biochemistry methodologies often include protocols where the detection and quantitation of proteins or nucleic acids is often desirable but where current methods are impractical. For example, the concentration of purified DNA in various buffers or in water is often measured spectrophotometrically at 260 nm using the extinction coefficient of A.sub.260 =1 for a 50 .mu.g/ml solution of double-stranded DNA or for a 40 .mu.g/ml solution of single-stranded DNA. Purified or crude DNA samples can also be measured spectrophotometrically using various assays such as diphenylamine or by fluorescence emitted by the binding of a minor groove binding dye diamidinophenylindole. These methods require several micrograms of DNA and do not distinguish between linear and closed circular DNA. Ethidium bromide staining of agarose gels containing DNA (50-100 ng) is routinely used in a typical molecular biology lab, but estimation of the concentration of stained DNA is complicated by differences in the kinetics of dye binding to various forms of DNA. Southern and dot blot techniques are useful for qualitative analysis, but are imprecise in quantitation of the target molecule due to the large variations in the efficiencies of a multistep procedure.

Brief Summary Text (10):
Accordingly, there is a need for methods of detecting and/or quantifying the presence and amount of nucleic acid molecules and proteins. There is a need for methods of detecting and/or quantifying the presence and amount of linear nucleic acid molecules in samples containing circular plasmids. There is a need for methods of detecting and/or quantifying the presence and amount of proteins contaminating samples containing nucleic acid molecules. There is a need for methods of detecting and/or quantifying the presence and amount of nucleic acid molecules contaminating samples containing protein molecules. There is a need for methods of measuring the amount of proteins or nucleic acid molecules present in a sample.

Detailed Description Text (2):
As used here, the term "biological polymer" is meant to refer to proteins and nucleic

acid molecules including DNA and RNA molecules.

Detailed Description Text (7):
According to the invention, ATP-dependent enzymes, i.e. enzymes that use ATP as a source of energy, are employed in reactions that digest or otherwise process proteins or nucleic acids (biological oligopolymers). The ATP-dependent enzyme which specifically cleaves or otherwise processes a biological polymer is combined with the sample containing the biological polymer. When the enzyme processes the biological polymer, it uses energy in the form of converting ATP into ADP. The amount of ADP generated in such a reaction is dependent on the rate for the reaction. The rate of the reaction is dependent on, among other factors, the amount of biological polymer present. Thus, the amount of ADP generated is correlated to the amount of biological polymer present in the sample.

Detailed Description Text (60):
Nucleic acids: Bacteriophage MS2 RNA, ribosomal RNA and transfer RNA were of the highest quality and were obtained from Boehringer Mannheim Biochemicals. The plasmid DNA was pUC4K from Pharmacia Biotech. Lambda DNA digested with the restriction enzyme HindIII was obtained from New England Biolabs.